NORGEN
BIOTEK CORPORATION
DNA PURIFICATION KITS
Plasmid MiniPrep Kit
PCR Purification Kit
DNA Gel Extraction Kit
A User Guide For Spin-Column Chromatography
©copyright 2005

Technical Support:

- techsupport@norgenbiotek.com
- 1.866.667.4362 (North America)
- 905.227.8848

Orders:

- orders@norgenbiotek.com
- 1.866.667.4362 (North America)
- 905.227.8848
- Fax: 905.227.1061

Precautions and Disclaimers

User must determine the suitability of the product for their particular use. These kits are intended for research purposes only and not for human or drug use. The kits are not designed for diagnostic purposes. MSDS sheets are available upon request.

General Introduction

The Norgen DNA Purification Kits (Plasmid DNA MiniPrep, PCR Purification, and DNA Gel Extraction) are designed for rapid purification using spin-column chromatography technology. At the heart of the technology is the newly introduced chromatography resin, silicon carbide (or SiC). Known for its unmatched material strength and durability, SiC is decidedly an important development for the increasingly popular spin-column format for rapid analytical purification.

As a new chromatography resin, SiC has proven to be versatile as it can be used in a wide variety of applications in DNA purification. Supercoiled plasmid DNA, PCR-amplified products, and DNA from agarose gels are among the many popular targets for purification used in molecular biology and biochemistry labs. Thus, Norgen has developed kits for the purification of DNA in these areas. In addition, SiC has proven to be a robust material for chromatography as it delivers consistent performance from batch to batch.

SiC is chemically inert and can tolerate extreme chemical and thermal treatments without affecting its binding and structural properties. It contains innate binding sites that are part of its crystalline nature and are not modified during various treatment conditions. The binding sites are not derivatized, hence leaching into the purified product cannot occur.

SiC reversibly binds DNA in a manner that depends on high ionic concentrations. Ions from chaotropic salts have proven effective for the process, which allows for separation and purification of nucleic acids from other cellular debris. In contrast to other systems that use chaotropes for binding DNA to solid phases, SiC requires sub-molar concentrations to provide maximal effect on binding. This feature is especially useful where high concentrations of chaotropes for extractions are specifically to be avoided.

Norgen's DNA isolation kits are valuable tools for providing substantial quantities of purified DNA that is compatible with all routine molecular biology techniques such as restriction enzyme digestions, DNA sequencing and cloning.

PLASMID MINIPREP KIT

Specifications

The Norgen Plasmid MiniPrep Kit is designed for rapid preparation of plasmid DNA from small batch cultures of *Escherichia coli* using spin column chromatographic separation. The kit contains sufficient materials for 50 preparations. The plasmid DNA is preferentially purified from other cellular components such as genomic DNA and RNA. Each spin column has a capacity of up to 21 ug plasmid DNA. Typical yield from a 1.5 mL culture for a high-copy plasmid is from 13 to 19 ug. Preparation time for 12 to 24 samples is approximately 30 minutes. The process does not require ethanol or other alcohols. The purified DNA is fully digestible with all restriction enzymes tested, and is completely compatible with manual or automated sequencing to achieve 95% to 100% accuracy. Purification of plasmids up to 13 kbp in size has been verified. The kit has a shelf-life of at least 6 months when stored as suggested.

Kit Components

Micro spin columns (assembled with their 2mL collection tube)
Elution Tubes (1.7 mL)
Resuspension Buffer
Lysis Solution
Binding Solution
RNase A
Wash Solution
Elution Buffer
User Manual
Short protocol card

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 6 months in their unopened containers. The **Resuspension Buffer** should be stored at 4°C upon addition of RNAse A enzyme.

Precautions and Disclaimers

User must determine the suitability of the product for their particular use. The kit is intended for research purposes only and not for human or drug use. The kit is not designed for diagnostic purposes. MSDS is available upon request.

The **Binding Solution** and **Wash Solution** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Ensure that lab coats and gloves are worn when working with this kit.

Materials Required But Not Provided

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g except where noted. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

Notes prior to use:

• Ensure that all solutions, except the **Resuspension Buffer**, are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.

• Take the entire amount of **RNAse A** and add it to the **Resuspension Buffer**. The label on the bottle has a box that can be checked to indicate that the RNAse A has been added. The solution can be stored for up to 6 months at 4°C.

• Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure.

Step 1. Lysate Preparation

a. Transfer 1.5 mL of bacterial culture to a microcentrifuge tube and centrifuge for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add 100 µL of the **Resuspension Buffer** (containing **RNAse A**; see note above) to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing. Incubate at room temperature for 5 minutes.

c. Add 100 µL of **Lysis Solution** to the cell suspension, cap the tube, and mix the contents by gently inverting the tube several times. Do not vortex as this will shear the genomic DNA. The suspension should become clear and viscous as the cells begin to lyse.

Continue mixing until the mixture becomes clear. If necessary, allow the solution to incubate at room temperature provided total incubation time is no more than 5 minutes. This step is also critical for the denaturation of cellular proteins and genomic DNA.

d. Add 300 µL of **Binding Solution** and immediately mix by inverting the tube several times. The solution will become turbid as insoluble particles from denatured materials start to form.

e. Centrifuge for 10 minutes to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

Step 2. Binding to Column

a. With a pipetman, transfer the lysate into a micro spin column attached to a 2mL collection tube. Ensure that none of the white particulates from step 1e are transferred onto the column. Cap the column, and then centrifuge the unit for one minute.

b. After centrifugation, separate the spin column from its collection tube. Discard the flowthrough, and reassemble the spin column with its collection tube.

Step 3. Washing Bound DNA

a. Apply 500 µL of the **Wash Solution** to the column, and centrifuge the unit for one minute.

b. Discard the flowthrough and reassemble the column and its collection tube.

c. Repeat steps 3a and 3b.

d. After the final wash, spin the reassembled unit for one additional minute to remove residual liquid. After centrifugation, separate the unit and discard the 2mL collection tube.

Step 4. Elution of Clean DNA (See note on extended elution below if a variable speed microcentrifuge is available)

a. Assemble the spin column (with DNA bound to the resin) with a fresh 1.7mL **Elution tube** included with the kit.

b. Add 25 µL of **Elution Buffer** to the center of the resin bed. It is important to place the **Elution Buffer** directly over the resin, and not on the side of the column, as this will decrease the DNA recovery. Centrifuge for one minute to elute the bound DNA.

c. Apply an additional 25 µL of **Elution Buffer** to the column and centrifuge for one minute. You will now have eluted the bound DNA in a total of 50 µL.

d. (Optional): An additional elution may be performed if desired. Another 50 µL of **Elution Buffer** may be added to the column and centrifuged for one minute into a new microcentrifuge tube. *Hint:* Separate the optional elution into a different centrifuge tube to avoid diluting the DNA solution in the first elution.

Extended Elution (alternate to steps 4b to 4d): For maximum recovery of bound DNA, elute with 50 µL of **Elution Buffer**. Centrifuge at 2,000 rpm for 2 minutes. Spin again for an additional minute at maximum speed (14,000 rpm).

Troubleshooting Guide for Plasmid MiniPrep Kit

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Plasmid did not propagate	Ensure that the appropriate growth medium, supplements and antibiotics were used for the host cell and plasmid of interest.
	Starting cell culture was old	Old bacterial cells are a poor source of plasmid DNA. Test-tube cultures grown overnight should be used immediately. Prolonged incubation or storage of culture in the fridge almost guarantees poor results.
	Lysate was prepared incorrectly	The **Lysis Buffer** may have formed precipitates. Warm and mix gently before use.
	Cell resuspension was incomplete	Pelleted cells should be completely resuspended in the **Resuspension Buffer.** Do not add **Lysis Solution** until a homogeneous suspension is obtained.
	Proper **Elution Buffer** was not used	The provided **Elution Buffer** has been optimized for high elution recoveries. If water is used, ensure that the pH is between 7 and 8.
	Elution Buffer was not placed directly over the resin.	It is important that the **Elution Buffer** be placed directly over the resin to ensure uniform passing of the buffer through the resin. Do not pipette the **Elution Buffer** onto the side of the column.
DNA does not perform well in downstream applications	DNA was not washed twice with the provided **Wash Solution**	Traces of salt from the binding step may remain in the sample if the column is not washed twice with **Wash Solution.** Salt may interfere with downstream applications, and thus must be washed from the column.
	A different **Elution Buffer** was used	If a different **Elution Buffer** other than the one provided in the kit was used, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

Frequently Asked Questions

1. What is the binding capacity of the columns?

   The saturating binding capacity of the column is approximately 21 µg of plasmid DNA.

2. Can recovery be increased further?

   Performing an optional elution with 50 µL of the **Elution Buffer** can increase the total amount of recovered DNA. This elution can either be kept in a separate tube in order to avoid dilution of the initial elution, or it can be collected into the same tube as the first elution.

3. How can DNA recovery be assessed?

   Yields of DNA recovered using the Plasmid MiniPrep kit can be determined by gel analysis. Run a portion of the eluted DNA on an agarose gel alongside known quantities of DNA as found in quantitative DNA molecular weight markers (see Norgen's line of DNA Molecular Weight Markers at www.norgenbiotek.com). By visual comparison with the known standards, or more accurately with gel densitometry, the mass of DNA present in the unknown can be established. Determinations using UV absorbance at 260 nm are not recommended.

4. How is RNA removed by the Plasmid MiniPrep Kit?

   The comparatively higher binding of plasmid DNA compared to RNA under high salt allows differential purifcation of DNA. Additionally, the **Resuspension Buffer** is supplemented with RNase A to allow breakdown of contaminating cellular RNA. The RNase is active during the resuspension step.

5. Why did the bacterial genomic DNA not purify with the plasmid DNA?

   The genomic DNA is denatured in the process and becomes part of the insoluble pellicle that is cleared away.

6. What sizes of plasmids can be isolated using the Plasmid MiniPrep kit?

   Plasmids up to 13 kbp in size may be isolated using the Plasmid MiniPrep kit.

7. Is it possible to elute the plasmid DNA in water?

Water may be used for the elution of the plasmid DNA provided the pH is not lower than 7. If this step is taken, ensure that the water used is molecular biology grade (deionized, salt-free, neutral pH and sterile).

8. What is the composition of the kit's Elution Buffer?

The **Elution Buffer** contains 10 mM TrisHCl and 0.1 mM EDTA. Due to the low concentration of EDTA, cation-dependent enzymatic reactions are not inhibited.

9. What is the dark material that is packed in the spin columns?

The dark chromatography material is silicon carbide (SiC). It is processed using proprietary methods to enable reversible binding of nucleic acids.

10. Can the columns be re-used?

Columns are designed for single use only. This minimizes sample carryover.

11. How should I store the Plasmid MiniPrep Kit solutions?

All solutions should be kept tightly sealed and stored at room temperature. Once RNase A has been added to the **Resuspension Buffer,** however, the solution should be stored at 4°C. All other reagents should remain stable for at least 6 months in their unopened containers. Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.

12. What are the maximum and minimum sample volumes that I can load onto the column?

We recommend that no more than 1 mL of sample be loaded onto the column. This ensures that the tip of the column does not touch the flow-through in the collection tube. The minimum load volume should be at least 100 µL.

13. What are the minimum and maximum amounts of DNA that I can load onto the column?

We recommend between 2 µg and 21 µg of DNA be loaded onto the column in order to obtain high recovery.

14. What are the highest and lowest recommended centrifuge speeds for the column?

We recommend a speed between 10,000 and 14,000 rpm in microcentrifuges (maximum 15,000 x g). Speeds below 10,000 rpm may be insufficient to completely move the liquid phase through the resin bed within the indicated centrifugation time. Additional spinning times may be required to remove this liquid.

15. What is the typical amount of DNA that I can expect to recover with the Plasmid MiniPrep Kit?

Depending on the plasmid copy-number, one can expect a yield of up to 21 µg of plasmid DNA with multiple elutions.

16. Can I autoclave the columns?

The columns are not designed to be autoclaved.

17. How can I tell if my DNA bound correctly to the column?

Save the flowthrough from the binding step, and run a sample on an agarose gel. If DNA can be detected in the flowthrough, then binding is not occurring efficiently. Since binding is dependent on both pH and salt concentrations, it is important that the correct amount of binding buffer was added to the sample prior to loading onto the column.

PCR PURIFICATION KIT

Specifications

The Norgen PCR Purification Kit is designed for rapid purification of PCR amplification products from reaction mixtures using spin columns. The kit contains sufficient materials for 50 preparations. PCR-amplified DNA ranging in size from 100 bp up to 15,000 bp can be purified using the kit. The typical purification yield is up to 95%. PCR byproducts including primers, dimers, residual enzymes, unincorporated nucleotides, salts and mineral oil used to overlay the reaction are efficiently separated from the DNA. The purified PCR products are fully compatible with restriction enzyme digestion, ligation into vectors, labeling and sequencing. In addition, the kit can be used as an alternative to organic extraction and ethanol precipitation to clean up various enzymatic reactions. Preparation time for 12 to 24 samples is about 15 minutes. The kit has a shelf-life of at least 6 months at room temperature.

Kit Components

- Micro spin columns (inserted in 2mL collection tube)
- Elution Tubes (1.7 mL)
- Binding Solution
- Wash Solution
- Elution Buffer
- User Manual
- Short protocol card

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 6 months in their unopened containers.

Materials Required But Not Provided

- Benchtop microcentrifuge
- 95% Ethanol (do not use denatured ethanol)
- 4M guanidine hydrochloride (optional)

Precautions and Disclaimers

User must determine the suitability of the product for their particular use. The kit is intended for research purposes only and not for human or drug use. The kit is not designed for diagnostic purposes. MSDS is available upon request.

The **Binding Solution** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Ensure that lab coats and gloves are worn when working with this kit.

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

Notes prior to use

• Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.

• Before starting the procedure, prepare a working concentration of the **Wash Solution** by adding 45 mL of 95% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution.** This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that the ethanol has been added. Do not use denatured alcohol, as this can lead to precipitation of salts.

• When pipetting any liquid onto the column, it must be applied to the center of the column to ensure even distribution and to maximize contact with the resin during centrifugation. This is particularly important when processing very small volumes (less that 100 µL).

1. Sample Preparation and Binding to Column

a. Add 5 volumes of **Binding Solution** directly to the tube containing the PCR reaction and mix well. Vortex and pulse-spin briefly in microcentrifuge to aid in mixing.

Note: It is not necessary to remove the mineral oil from the PCR sample, as it will be removed during the purification process.

b. Apply the sample to a column and centrifuge for one minute. The maximum volume that the reservoir can accommodate during each spin is 1 mL. If the sample volume exceeds this, repeat spin as necessary until the entire sample has been processed.

c. Discard the flowthrough, and reassemble the spin column with its collection tube.

2. Washing Bound DNA

Note: An additional wash step is necessary to remove primer-dimers when present in large amounts in a PCR reaction product. After the binding step, wash the column with 500 µL of a 4M Guanidine Hydrochloride wash (to be provided by the user). Then proceed with the protocol below.

a. Apply 500 µL of **Wash Solution** to the column and centrifuge for one minute.

b. Discard the flowthrough and reassemble the column and its collection tube.

c. Repeat steps 2a and 2b.

d. Spin column for one additional minute in collection tube to remove any remaining liquid.

3. Elution of Clean DNA

a. Assemble the column with a fresh 1.7mL **Elution tube** provided with the kit.

b. Add 25 µL of **Elution Buffer** to the center of the resin bed, and centrifuge for one minute. It is important that the **Elution Buffer** be placed directly onto the resin bed, and not onto the side of the column to obtain the best DNA recovery.

c. Apply an additional 25 µL of **Elution Buffer** to the column and centrifuge for one minute. You will now have eluted the bound DNA in a total of 50 µL. The recovered amount constitutes 80%-90% of the bound DNA.

Troubleshooting Guide for the PCR Purification Kit

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Binding of DNA to the column was inefficient	Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of **Binding Solution** was used for the volume of the PCR reaction.
	The appropriate amount of ethanol was not added to the **Wash Concentrate.**	The **Wash Solution** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution** was prepared using the correct amount of 95-100% ethanol.

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	**Binding Solution** was not completely removed in the wash step.	Traces of salt left on the column from the binding step may interfere with the elution of the DNA. Ensure that the column is washed twice with the **Wash Solution.**
	Proper **Elution Buffer** was not used	The provided **Elution Buffer** has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure that the pH is around 8.
	Elution Buffer was not placed directly onto the resin.	It is important that the **Elution Buffer** be placed onto the center of the resin bed, as this helps to increase recovery by ensuring an even passing of the buffer through the resin. Do not pipette the **Elution Buffer** onto the side of the column.
DNA does not perform well in downstream applications	Insufficient washing of resin with bound DNA	Traces of salt from the binding step may remain in the sample if the column is not washed at least twice with the **Wash Solution.** Salt may interfere with downstream applications, and thus must be washed from the column.
	Incomplete removal of **Wash Solution**	Ensure that the column is spun for 1 minute after the second wash step, in order to completely dry the column. Traces of **Wash Solution** may remain in the eluted sample otherwise, and interfere with subsequent enzymatic reactions.
	Eluate contains primer-dimers	Washing the column with a 4M GuHCl wash after binding the DNA will help to remove additional primer-dimers. Proceed with the regular washes after this optional wash. Alternatively, the elution can be recycled through the column in order to remove additional primer-dimers, although this may lead to a loss of some DNA.

Frequently Asked Questions

1. Does the kit remove primer-dimers?

The kit typically removes between 75 and 85% of primer-dimers when the optional 4M guanidine hydrochloride wash is used. If it is important that a greater amount of dimers is removed, it has been found that recycling the elution through the column a second time results in 80-90% dimer removal. However, some loss of DNA is also observed when the elution is recycled.

2. Does the kit remove mineral oil?

The kit is capable of removing mineral oil that is commonly used in PCR reactions. It is not necessary, therefore, to remove it prior to binding to the column.

3. What is the binding capacity of the columns?

The binding capacity of the column is approximately 10 µg of DNA.

4. Can recovery be increased further?

Recovery can be increased by performing an additional elution using 50 µL of the **Elution Buffer.** This elution can either be kept in a separate tube in order to avoid dilution of the initial elutions, or it can be collected into the same tube as the first elutions. However, for most fragments 80-90% of DNA is eluted in the first elutions.

5. How can DNA recovery be assessed?

The best method for assessing yields of DNA recovered using the PCR Purification Kit is by gel analysis. Run a portion of the eluted DNA on an agarose gel alongside known quantities of DNA such as commercial DNA markers (see, for example, Norgen's line of DNA markers at www.norgenbiotek.com). By visual comparison with the known standards, or more accurately with gel densitometry, the mass of DNA present in the unknown can be estimated. Determinations using UV absorbance at 260 nm are not recommended.

6. What is the dark material that is packed into the spin columns?

The dark chromatography material is silicon carbide (SiC). It is processed using proprietary methods for maximum binding of nucleic acids.

7. Can the columns be re-used?

Columns are designed for single use only. This minimizes sample carryover.

8. How should I store the PCR Purification Kit solutions?

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 6 months in their unopened containers. Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.

9. What are the maximum and minimum sample volumes that I can load onto the column?

We recommend that no more than 1 mL of sample be loaded onto the column, to ensure that the tip of the column does not rest in the flowthrough that is caught in the collection tube. The minimum load should be at least 100 µL to completely cover the resin bed for binding.

10. What is the highest and lowest recommended centrifuge speeds for the column?

We recommend a speed between 10,000 and 14,000 rpm in microcentrifuges (maximum 15,000 x g). Speeds below 10,000 rpm may be insufficient to completely move the liquid phase through the resin bed. Additional spinning times may be required to remove this liquid.

11. Can I autoclave the colums?

No. The columns are not designed to be autoclaved.

12. How can I tell if my DNA bound correctly to the column?

Save the flowthrough from the binding step, and run a sample on an agarose gel. If DNA can be detected in the flowthrough, then binding is not occurring efficiently. Since binding is dependent on both pH and salt concentrations, it is important that the correct amount of binding buffer added to the sample prior to loading onto the column. It is also important that the binding capacity of the column is not exceeded.

DNA GEL EXTRACTION KIT

Specifications

The Norgen DNA Gel Extraction Kit is designed for rapid purification of DNA fragments that have been fractionated on agarose gels. The kit contains sufficient materials for 50 preparations. DNA fragments with a size range of 100 bp to 10,000 bp can be purified using the kit. The typical purification yield is up to 90%. The large reservoir of the columns allows processing of up to 250 mg of gel slice in a single spin. The recovered DNA is free from agarose and other impurities and is compatible with restriction enzyme digestion, ligation into vectors and sequencing. Preparation time for 12 to 24 samples is about 30 minutes. The kit has a shelf-life of at least 6 months at room temperature.

Kit Components

Micro spin columns (inserted in 2mL collection tube)
Elution Tubes (1.7 mL)
Binding Solution
Wash Solution
Elution Buffer
User Manual
Short protocol card

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 6 months in their unopened containers.

Materials Required But Not Provided

- Benchtop microcentrifuge
- 95% Ethanol
- Isopropanol

Precautions and Disclaimers

Users must determine suitability of the product for their particular use. The kit is intended for research purposes only and not for human or drug use. The kit is not designed for diagnostic purposes. MSDS is available upon request.

Ensure that lab coats and gloves are worn when working with this kit. Protective eyewear should be worn when working with UV light.

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

Notes prior to use

- Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.

- Before starting the procedure, prepare a working concentration of the **Wash Solution** by adding 45 mL of 95% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that the ethanol has been added. Do not use denatured alcohol, as this can lead to precipitation of salts.

1. Excising DNA from Gel

a. Run DNA fragment of interest on agarose gel.

Note: It is recommended that fresh buffer be used for running the gel. A used one may have its buffering capacity exhausted and may subsequently cause reduced yields.

b. Excise fragment from agarose gel using a scalpel or razor blade. Remove as much excess agarose as possible. Minimize exposure of DNA to UV light.

c. Place the excised agarose into a sterile and pre-weighed 1.5 mL microcentrifuge tube.

2. Sample Preparation

a. Determine the weight of the gel slice.

b. Add 3 volumes of the **Binding Solution** to 1 volume of gel (i.e., it is assumed that the gel has the same density as water so that 100 mg of gel occupies the same volume as 100 µL of **Binding Solution**).

Note: For example, add 300 µL of **Binding Solution** to a 100 mg gel slice. For gels made with greater than 2% agarose, add 6 volumes of **Binding Solution.** For larger gel slices (greater than 300 mg) cut gel into smaller pieces to facilitate melting.

c. Incubate at 55°C for up to 10 minutes, or until completely dissolved. (Please see Table A for a time guideline.) Vortex every 2 to 3 minutes to assist in dissolving. It is important to dissolve the gel slice completely.

d. Once gel slice is completely dissolved, add 1 gel volume of isopropanol and mix.

Note: For example, if the gel slice is 100 mg, add 100 µL of isopropanol.
Do not centrifuge during this step of the protocol.

3. Binding DNA to Column

a. Apply the sample to the column and centrifuge for one minute. The maximum volume that the reservoir can accommodate during each spin is 1 mL. If the sample volume exceeds this, repeat spin as necessary until the entire sample has been processed

b. Discard the flowthrough, and reassemble the spin column and its collection tube.

4. Washing Bound DNA

a. Apply 500 µL of **Wash Solution** to column and centrifuge for one minute.

b. Discard the flowthrough and reassemble column and collection tube.

c. Repeat steps 4a and 4b.

d. Spin the column unit for one additional minute to completely remove any liquid.

5. Elution of Clean DNA

a. Assemble the column with one of the provided 1.7 mL **Elution tubes.**

b. Add 30 µL of **Elution Buffer** to the center of the resin bed and centrifuge for one minute. It is important that the **Elution Buffer** be placed directly onto the resin bed, and not onto the side of the column as this will decrease the efficiency of DNA recovery.

c. (Optional): An additional elution may be performed if desired. Another 30 µL of the **Elution Buffer** should be added to the column and centrifuged for one minute into a new elution tube to avoid diluting the initial elution.

Note: 80 to 90% of bound DNA should elute with the first elution, however, additional DNA can be obtained with the second elution.

Table A: Separation of DNA in Gels Containing Different Concentrations of Agarose

Amount of Agarose in Gel (%w/v)	Efficient Range of Separation of Linear DNA Molecules (kbp)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0.2-3
2.0	0.1-2

Table B: Time Required to Melt 100mg of Gel Slices of Varying Agarose Concentrations at 55°C

Percent Agarose	Three (3) volumes Binding Solution	Six (6) volumes of Binding Solution
1 %	4 minutes	4 minutes
2 %	4 minutes	4 minutes
3 %	NR	8 minutes
4 %	NR	10 minutes

NR = Not Recommended. Norgen does not recommend the use of 3 volumes of Binding Solution for greater than 2% gels since resulting melted gel slice is viscous and will hinder the flow of solutions through spin columns.

Troubleshooting Guide for DNA Gel Extraction Kit

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	pH of the electrophoresis buffer was too high	Ensure that fresh running buffer is used for electrophoresis. When the buffer is re-used, it often exhibits increased pH and may subsequently reduce yields.
	Gel slice was not completely melted in the **Binding Solution**	The gel slice should be incubated at 55°C until completely dissolved. The slice should be vortexed every 2 to 3 minutes to assist dissolving.
	The appropriate amount of ethanol was not added to the **Wash Concentrate**	The **Wash Solution** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution** was prepared with the correct amount of 95% ethanol.
	Proper **Elution Buffer** was not used	The provided **Elution Buffer** has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure that the pH is around 8.
	Elution Buffer was not placed directly onto the resin.	It is important that the **Elution Buffer** be placed directly onto the resin, as this helps to increase recovery by ensuring an even passing of the buffer through the resin. Do not pipette the **Elution Buffer** onto the side of the column.
	Binding of DNA to the column was inefficient	Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of **Binding Solution** was used for the weight of the gel slice.
	Binding Solution was not completely removed in the wash step.	Traces of salt left on the column from the binding step may interfere with the elution of the DNA. Ensure that the column is washed twice with the **Wash Solution.**

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	Incomplete removal of **Wash Solution**	Ensure that the column is spun for 1 minute after the second wash step, in order to completely dry the column. Traces of **Wash Solution** may remain in the eluted sample otherwise, and interfere with subsequent enzymatic reactions.
	DNA was not washed twice with the provided **Wash Solution**	Traces of salt from the binding step may remain in the sample if the column is not washed twice with **Wash Solution.** Salt may interfere with downstream applications, and thus must be washed from the column.

Frequently Asked Questions

1. Can the kit be used for various percentages of agarose gels?

Yes. For gels made with 2% or less agarose, 3 volumes of **Binding Solution** should be used. For gels higher than 2%, use 6 volumes of **Binding Solution.**

2. Can this kit isolate DNA from TBE gels?

The kit can be used for TBE gels. However, it is important to ensure that the pH after dissolving agarose does not become basic.

3. What is the binding capacity of the columns?

The binding capacity of the column is approximately 10 µg of DNA.

4. Can recovery be increased further?

Recovery can be increased by performing an additional elution using 30 µL of the **Elution Buffer**. This elution can either be kept in a separate tube in order to avoid dilution of the initial elution, or it can be collected into the same tube as the first elution.

5. How can DNA recovery be assessed?

The best method for assessing yields of DNA recovered using the DNA Gel Extraction Kit is by gel analysis. Run a portion of the eluted DNA on an agarose gel alongside known quantities of DNA such as commercial DNA markers (see, for example, Norgen's line of DNA markers at www.norgenbiotek.com). By visual comparison with the known standards, or more accurately with gel densitometry, the mass of DNA present in the unknown can be estimated. Determinations using UV absorbance at 260 nm are not recommended.

6. What is the dark material that is packed into the spin columns?

The black chromatography material is silicon carbide (SiC). It is processed using proprietary methods to function as an ion exchange chromatography resin.

7. Can the columns be re-used?

Columns are designed for single use only. This minimizes sample carryover.

8. How should I store the DNA Gel Extraction Kit solutions?

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 6 months in their unopened containers. Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.

9. What are the maximum and minimum sample volumes that I can load onto the column?

We recommend that no more than 1 mL of sample be loaded onto the column, to ensure that the tip of the column does not rest in the flowthrough that is caught in the collection tube. The minimum load should be at least 100 µL to completely cover the resin bed for binding.

11. What is the highest and lowest recommended centrifuge speeds for the column?

We recommend a speed between 10,000 and 14,000 rpm in microcentrifuges (maximum 15,000 x g). Speeds below 10,000 rpm may be insufficient to completely move the liquid phase through the resin bed. Additional spinning times may be required to remove this liquid.

12. Can I autoclave the columns?

No. The columns are not designed to be autoclaved.

13. How can I tell if my DNA bound correctly to the column?

Save the flowthrough from the binding step, and run a sample on an agarose gel. If DNA can be detected in the flowthrough, then binding is not occurring efficiently. Since binding is dependent on both pH and salt concentrations, it is important that the correct amount of binding buffer be added to the sample prior to loading onto the column.

NORGEN BIOTEK CORPORATION
Ontario, Canada
toll free: 1.866.667.4362 · phone: 905.227.8848
fax: 905.227.1061 · www.norgenbiotek.com

P/N # 14405 Version 1.1